# Analyse phénotypique et génotypique de 30 patients ayant une épidermolyse bulleuse héréditaire de type jonctionnelle avec mutations du gène codant pour le collagène XVII, COL17A1

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# ANALYSE PHENOTYPIQUE ET GENOTYPIQUE DE 30 PATIENTS AYANT UNE EPIDERMOLYSE BULLEUSE HEREDITAIRE DE TYPE JONCTIONNELLE AVEC MUTATIONS DU GENE CODANT POUR LE COLLAGENE XVII, *COL17A1*.

## THESE D'EXERCICE EN MEDECINE

Par

Anne-Laure Hérissé

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# TABLES DES MATIERES

# ABREVIATIONS

EB : Epidermolysis Bullosa

JEB : Jonctional Epidermolysis Bullosa

DEB : Dystrophic Epidermolysis Bullosa

*COL17A1* : Genetic name of Collagen XVII

PCR : Polymerase Chain Reaction

DNA : Deoxyribonucleic acid

RNA : Ribonucleic acid

BMZ : Basement membrane zone

HD : Hemidesmosome

CCA : Congenital cutis aplasia

NC domain : Non Collagenous domain

X : PTC - Premature Termination Codon

SS : Splice site mutation

A : Ala - Alanine (amino acid)

E : Glu - Acide glutamique (amino acid)

G : Gly - Glycine (amino acid)

Y : Tyr - Tyrosine (amino acid)

L : Leu - Leucine (amino acid)

Q : Gln - Glutamine (amino acid)

R : Arg - Arginine (amino acid)

S : Ser - Serine (amino acid)

NA : Non Available

PMD : Psycho motor delay

Ins : Insertion mutation

Del : Deletion mutation

# INTRODUCTION

Inherited epidermolysis bullosa (EB) is a heterogeneous group of rare recessively inherited genodermatoses characterized by skin and/or mucosal fragility resulting in blistering. International consensus meetings on diagnosis and classification of EB have categorized four major EB types: simplex EB, junctional EB, dystrophic EB and Kindler syndrome based on the level of cleavage of the blister within the skin [1, 2 and 3]. Consistent with this classification, junctional epidermolysis bullosa (JEB) includes all subtypes of EB in which blisters develop within the dermal-epidermal junction of the integument, at the level of the lamina lucida of the basement membrane zone. JEB, which comprises a variety of generalized and localized subtypes, is associated with genetic mutations affecting one of the of six candidate genes expressing the components of the hemidesmosome-anchoring filament adhesion complex [2, 3, 4 and 7]. Collagen XVII, a hemidesmosome transmembrane component, consists of an N-terminal intracytoplasmic domain that binds to integrin ß4 and to the inner hemidesmosome plaque components plectin and BP230, a short transmembrane domain and a C-terminal extracellular domain with 15 collagenic domains and 16 non-collagenic domains. This ectodomain contains the binding sites for laminin 332, integrin α6 and collagen IV [4, 5, 6, 7 and 8]. Genetic mutations in the *COL17A1* gene that encodes collagen XVII result in either localized or generalized intermediate JEB. Recently, *COL17A1* mutations have been involved in autosomal dominant diseases, such as a dominantly-inherited epithelial recurrent erosion dystrophy (ERED)-like disease common in northern Sweden [9] and a novel form of random pitting, dental enamel hypoplasia described in USA [10]. Less than 100 JEB patients with clinical phenotype and molecular analysis have so far been reported in the literature [2, 3].

The aim of our study was to contribute to the establishment of genotype-phenotype profiles in patients with JEB associated with *COL17A1* genetic mutations.

# MATERIAL AND METHODS

## *1- Patients:*

All patients were diagnosed as JEB with collagen XVII alterations by immunofluorescence analysis of skin biopsies and/or molecular analysis of *COL17A1* genetic mutations in our center between June 1993 and July 2015. When available, medical and biological records were collected with the help of a standardized questionnaire (age at diagnosis, extent of skin lesion, mucosal involvement, cutaneous infections, skin cancers, nail dystrophy, alopecia, digestive problems and malnutrition, orthopedic abnormalities, geographical origin and social insertion).

## *2- Immunofluorescence analysis:*

The level of blister formation and protein expression were determined using frozen skin biopsies. Monoclonal antibodies were HD121 to plectin (gift of Dr Owaribe, Japan), GoH3 to integrin α6 (kind gift of A. Sonnenberg, Amsterdam, Netherlands); 3E1 (Gibco BRL Life Technologies, Cergy Pontoise, France) to integrin ß4; 233 (kind gift of Dr Owaribe, Japan) specific to the collagen type XVII; GB3 to laminin-5 (our production), LH7:2 (Cymbus Biotechnology, Chandlers Ford, UK) to collagen type VII. Secondary antibodies were fluorescein isothiocyanate conjugated goat antimouse Ig (Dako, Trappes, France), goat antirat IgG (Cappel, ICN Biomedicals, Orsay, France), or swine antirabbit Ig (Dako, Ville, France) [11]. The tissue sections were examined using an epiluminescence Zeiss Axiophot microscope.

## 3- *Molecular analysis of COL17A1 gene*:

Genomic DNA was isolated from peripheral blood of the patients, their parents when available and unrelated controls using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). All exons of the *COL17A1* gene were amplified by Polymerase Chain Reaction (PCR) using 50–100 ng of genomic DNA as template in 25-ul reactions with 20pmol each of flanking intronic primers and 0.5 U Taq-Polymerase (Life Technologies, St. Aubin, France). The PCR products were purified using QIAGEN PCR Purification Kit (Qiagen, Hilden Germany), then subjected to cycle sequencing with Big Dye Terminator Cycle Sequencing Kit (according to manufacturer's recommendations, ABI-Life Technologies, Carlsbad, CA USA) and analyzed on an ABI PRISM 310 Genetic Analyser (ABI-Life Technologies, Carlsbad, CA USA). The designation of mutations and numbering of the nucleotides and amino acids was performed according to nomenclature recommendations.

# RESULTS

## *1- Patients:*

Thirty patients were included, sex ratio = 1, middle age 13 years old (from 0 to 74 years) (Table 1) divided into: eleven adults over 18, seven teenagers (from 13 to 17 y-o), four children (from 3 to 12 y-o) and eight infants (from 0 to 2 y-o). Fifteen of them were products of non-consanguineous unions. Sixteen patients were from France, six from Maghreb, three from Italy, and one each from Spain, Cape Verde, Mali, Turkey and Arabic Emirates. Patients 22 and 23 were brothers. Diagnosis was established at birth for 16/30 patients, during childhood for 6/30 patients, in adolescence for 1/30 patients, in adulthood for 7/30 patients. Twenty-two patients presented a generalized form, one a late onset of the condition, the mutation of which has previously been published [12] and two a localized form. No clinical data were available for five patients. The first symptoms appeared in the neonatal period for 19/25 patients, between the first month of life and 2 y-o for 5/25 patients, in childhood for one patient. Three patients died during their first year of life. Detailed clinical data were available only for 25 patients.

## *2- Clinical features* *(Table 1 and Fig 1)*:

### *A. High frequency of extensive congenital cutis aplasia:*

Fourteen of 25 patients presented with congenital cutis aplasia (CCA). Among these 14 patients, CCA localized on the four limbs (hands, feet and/or legs) in 10 cases, on the lower limbs in 2 patients and once on the hands. Hospitalization in neonatology department was required for all patients for dressings and analgesic treatments. CCA was associated with the most severe forms of generalized intermediate JEB with important skin fragility and nail dystrophy. Three patients presenting CCA died in their first months of life. No functional sequelae were reported after complete healing of CCA, except for patient 9 who presented synechia of toes on his/her left foot.

### *B. Variable evolution of dermatological condition over time:*

Phenotypic analysis showed a wide range of disease severity during infancy. In 16 patients the phenotype was severe, with exacerbated skin fragility, generalized blistering, CCA, severe mucosal involvement, nail and dental dystrophy, leading to demise in three cases. In 6 other patients, JEB was moderate with occasional blistering, mucosal involvement and inconstant dental and nail dystrophy. Finally, 3 patients were asymptomatic during infancy (Fig 1). Analysis of the disease course provided interesting information. Global evolution of the disease was favorable for 13 out of 25 patients, with progressive improvement of their skin condition, good social integration and personal development (marriage and children, employment, sport activity and autonomy for care). This was despite the fact that four of these patients suffered from a severe phenotype during infancy. In contrast to improvement of skin involvement, mucosal lesions persisted in adulthood in all patients, without noticeable improvement. In adulthood, uro-genital lesions, as anal fissures or urethral stricture, were the most common mucosal lesions observed, as well as crusted chronic lesions in the nose and buccal lesions. Evolution was unfavorable for 12 patients, including the three patients that died during their first months of life. Three adults suffered from malnutrition, social and personal difficulties. Six children had psycho-motor development and/or delayed growth, malnutrition, or extensive skin involvement with time-consuming dressings. Twenty-three among the 25 patients presented nail dystrophy, 15 had universal alopecia beginning in adolescence and 13 suffered from dental dystrophy, including enamel defects, caries and/or parodontitis. Chronic constipation was observed in 6 cases and renal failure in one (patient 5). No pyloric atresia, esophageal stricture or cancer was reported in our cohort.

### 3- *Immunofluorescence analysis (table 1):*

This was performed on skin biopsies obtained from 29 patients. It showed cleavage within the basal membrane zone, which is characteristic of JEB, with decreased fluorescent staining of collagen XVII at the roof and the floor of the blister in 4 patients (patients 15, 16, 24 and 25), negative staining in 25 patients, compared to healthy controls. In accordance with the diagnosis of JEB, collagen VII and laminin-5 were expressed and mapped to the blister floor; keratins and plectin to the blister roof and integrin α6β4 both to the floor and roof of the blister.

### *4- **Direct sequencing of genomic DNA** (Table 1 and Fig 2):*

Sequencing of genomic DNA identified 34 mutations in *COL17A1* gene in 28 patients, of which, to our knowledge, 24 are novel. No mutation was found after sequencing all exons of *COL17A1* gene for patient 25 and analysis is still ongoing for patient 9. Homozygous gene mutations were found in 19/28 patients and heterozygous mutations in 9/28 patients. Consanguineous couples were reported in 14 of 28 patients. Eleven of 34 mutations are nonsense mutations, 3 insertion and 9 deletion mutations predicted to lead to premature termination codon (PTC); 4 missense mutations and 6 splice site mutations. Splice site mutations concerned in 7 patients (patients 12, 20, 22, 23, 26, 28, and 29) for whom 3 mutations were novel. In patient 24, the change of c.3198c/t hampers the recognition of the natural 3' splice site of exon 46 which is 11 nucleotides downstream at c.3208. The exon-intron splice site is anticipated and occurs at c.3192 which results in the deletion of 16nt, and corresponds to p.Val1065_1070delLeufs.

Three mutations were found to be recurrent in our European patients. Mutation p.Arg1226X in exon 51 found in two unrelated patients from France and Spain (patients 6 and 19), mutation p.Gly446Ser in exon 17 found in two unrelated French patients (patients 20 and 21), and mutation p.Tyr207X in exon 10 found in two unrelated French patients (patients 11 and 15).

We observe that 19 of 34 mutations are localized in a collagenous domain of the protein, of which 8 mutations are in Col15 domain (Fig 2). Nine mutations are localized in binding site regions of which 8 are at the level of laminin-5 binding site and one at the level of plectin-BP230 binding site. Seventeen are localized in non-collagenous domain (NC) of which 2 are localized in NC16a ectodomain.

## *5- Phenotypic-genotypic correlations:*

The wide spectrum of clinical phenotypes, the type and location of mutations in the *COL17A1* and the low rate of recurrent mutations explain the difficulties to define phenotype-genotype correlations. As expected, patients with glycine or arginine substitutions, in particular in the homozygous state, had milder phenotypes. Similarly, patient 16, homozygous for an in-frame 3-nucleotides insertion presented with a mild phenotype. Patients with splice site mutations also suffered from mild phenotypes, except for one patient (patient 12) who was a heterozygous composite with a nonsense mutation. As expected, patients with two nonsense mutations or mutations leading to premature stop codons usually were affected by the most severe phenotypes.

Patients 6 and 19 were compound heterozygotes for the recurrent mutation p.Arg1226X [17]. Patients 6, carrier of a PTC mutation in exon 26, died 3 month old; patients 19, carrier of a nonsense mutation in exon 52, presented a mild phenotype. Two unrelated French patients (patients 20 and 21) were compound heterozygotes for missense mutation p.Gly446Ser in exon 17, which resulted in mild phenotypes and favorable clinical course. Second mutations were a splice site mutation and a PTC mutation in exon 21 and 46, respectively. Both patients suffered from dental and nail involvement, mucosal involvement localized in buccal mucosa and uro-genital tract. The adult patient showed alopecia, which did not affect the adolescents; no CCA was observed in the neonatal period.

Two other unrelated French patients (patients 11 and 15) were homozygous for the PTC mutation p.Tyr207X in exon 10. This mutation localized in the intracellular domain of collagen XVII at level of the plectin-BP230 binding site. Patient 11, 54 years old with mild phenotype, suffered from dental abnormalities, alopecia and social difficulties, while in patient 15, 10 month old, the JEB phenotype was mild with limited skin involvement.

Three additional patients were homozygous for PTC mutations affecting the intracellular domain of collagen XVII. In patients 5 and 7, PTC mutations in exon 3 and 7, respectively, resulted in severe phenotypes, which was fatal for patient 5 who deceased in the first month of life while resulted in delayed psychomotor development and growth in patient 7. Interestingly, these severe manifestations

were linked to PTC localized upstream the binding domain for plectin-BP230, while PTC within in exon 17 located downstream the binding domain for plectin-BP230, just before the transmembrane domain, caused a moderate phenotype in Patient 17).

Eight mutations localized in the larger (col15) collagenic domain of collagen XVII. Patient 2 and 27 were homozygous for glycine substitutions, five patients (n°3, 4, 6, 20 and 29) had PTC mutations, while patient 28 presented splice site mutations. Patients 2 and 3 showed very localized phenotypes, Patients 4 and 6 severe phenotypes, while patient 20 displayed a mild phenotype. No clinical data were available for patients 27, 28 and 29.

In five patients the genetic mutations affected one of the six exons encoding for the 6 C-terminal collagenic domains, where the binding sites for laminin-5 and collagen IV are predicted. Patient 1 had the already reported p.Arg1303Glu missense mutation leading to EBJ late onset. In the four other patients, the PTCs were either homozygous or compound heterozygous with distinct PTC mutations which resulted in various JEB phenotypes: Patient 6 presented with a lethal generalized intermediate JEB; in Patients 13 and 14 JEB was generalized with malnutrition, and moderate in patient 19 with a favorable clinical course.

Finally, patient 24, with a mild phenotype, was compound heterozygous for splice site mutation c.3198C/T in exon 46, which creates a cryptic donor site resulting in frame shift and downstream PTC, and mutation c.2972delT in exon 45, which also leads to a downstream PTC.

# DISCUSSION

The current canonical features of patients with JEB due to *COL17A1* mutations are skin fragility with generalized sero-hemorrhagic blisters, mucosal involvement, pigmentary changes, atrophic scars, universal alopecia, dystrophic nails and dental abnormalities [13, 14], which are also observed in most of our patients.

Our study provides interesting new clinical information. First, CCA seems to frequently occur in JEB associated with genetic mutations in *COL17A1*, because it affects more than 50% of our patients, with a peculiar involvement of the four limbs. On upper limbs the back of both hands were involved, reminiscent of the pattern of congenital suction blisters, but with deeper ulceration. Lower limb CCA was classical but without orthopedic sequelae as described for DEB patients. Interestingly, the presence of CCA was associated with the most severe phenotypes and skin fragility. Clinically CCA associated with mutation in *COL17A1* presented differently from the extensive CCA described in patients with JEB with pyloric atresia due to anomalies in plectin or integrin α6β4 [2, 15, 16], however no clear correlation with the genotype could be established.

Following the patients' clinical evolution with aging, in a majority of cases a progressive improvement of the skin involvement took place, which was not observed in mucosal involvements that constantly persisted. Accordingly, in adulthood, complications were frequently reported by patients (n=11), which included: mucosal complications (mainly oral and uro-genital involvement, and, at a lower level, also eyes and nose) and dystrophic nails. Universal alopecia and dental abnormalities were found in only half of patients, with no clear correlation between these two manifestations. The young age of our patients can explain such a low percentage, but in a recent article, Kiritsi et al [17] found similar results. Interestingly, Olague-Marchan et al [10] hypothesized that glycine substitutions in the *COL17A1* gene appear to give rise to a novel form of random pitting of dental enamel and hypoplasia. Half of the patients had acceptable social integration and personal development, according to the old denomination of the disease: generalized atrophic benign epidermolysis bullosa (GABEB).

Also, the wide range of phenotype severity in this disease is noticeable. In contrast to patients with favorable evolution of the condition, very severe forms affected three patients, who died in their first months of life: because of sepsis in two instances and renal failure in one case. Sepsis is the most frequent cause of death in patients with severe JEB [15, 18]. Patient 5 with renal failure presented a novel *COL17A1* mutation, c.56delT, which causes a frameshift of the *COL17A1* coding reading frame. Renal disease in generalized intermediate JEB has not been reported yet [2, 18 and 19]. On the contrary, 4 patients with severe neonatal presentation underwent favorable clinical evolution in adulthood. Seven other suffered from systemic complications with malnutrition, musculoskeletal limitations and poor social integration. No cancer or esophageal stricture was reported, as described in literature [16, 18, 19 and 20].

So far, less than 100 patients with genetic mutations in COLXVII have been described [12, 17, 21 and 22]. Confrontation of our results with the previously reported cases confirms the heterogeneity within the constellation of the known *COL17A1* mutations, in which recurrent mutations are rare and hot spot mutations are absent. This feature is contrast with what observed with other genes involved in inherited EB, such as *COL7A1* or *LAMB3*, which can also explain the rarity of this form of the condition. According with other authors, we found that nonsense mutations, insertions and deletions are the most frequent genetic alterations and that missense mutation more commonly localize in col15, the largest collagenic domain of the protein [13, 23, 24 and 25]. Kiritsi et al [17] suggested that most mutations occur in *COL17A1* exon 51 and 52, but our results fail to corroborate this hypothesis. We however confirm that p.Arg1226X is a recurrent European mutation [17]. The recurrent “Italian” p.Arg795X mutation was detected in a French composite heterozygous patient, but not in the four Italian patients of our cohort [17, 22]; neither did we found the recurrent p.Gly803X mutation reported by Kiritsi et al [17]. Mutation p.Arg1303Glu, appears to be recurrent, because it has been published by several teams and seems to be specific of the late-onset or localized JEB phenotype [12, 17 and 21].

Further genotype-phenotype correlations are difficult to establish, but a few considerations can be made in the light of our observations. Presence of a residual

immunofluorescence staining in is the skin biopsy is always associated with a mild phenotype [17]. Missense mutations, in particular those affecting the collagenic domain coll15, and splice site mutations seem to result in milder JEB phenotypes. We were unable to evaluate the possible presence of a dominant effect of missense mutations on the dental enamel in the patients' parents [10]. Similarly, in the case of mutation c.3198C/T mutation in exon 46, which is very similar to the recently published mutation c.3156C/T, we could not explore the patient's parents for possible epithelial recurrent erosion dystrophy [9]. In patients with a nonsense mutations or insertion/deletion mutations leading to PTC, the phenotype is usually severe; in this study, however, a few patients homozygous for a nonsense mutation displayed a mild clinical phenotype. Lack of biological material prevented us to verify whether such a mild phenotype could be explained by expression of a (partially) functional collagen XVII molecule consequent to alternative splicing mechanisms rescuing messenger RNA expression or by spontaneous revertant mosaicism, as reported in the literature [17, 18, 19 and 26]. On the contrary, distal *COL17A1* mutations lead to severe phenotype, which is presumably consequent to premature degradation of mutated messenger RNA and absence of protein expression in the skin, as shown for the recurrent mutation p.Arg1226X [17, 27 and 28].

In conclusion, this study underlines the high variability of phenotypic presentation of inherited JEB associated with genetic mutations in *COL17A1* that range from late JEB onset to severe clinical phenotypes resulting in premature demise of the patients. High frequency of CCA affecting the four limbs in severely affected patients is also reported, as well as a favorable evolution in a majority of patients of the skin involvements over time. We confirm that residual staining of collagen XVII in indirect immunofluorescence of skin biopsy is hallmark of a favorable prognosis. At the moment, however, and with the exception of extremely rare recurrent mutations, valid genotype-phenotype correlations cannot be established. Such correlations will require identification of additional cases of JEB and needs mRNA expression analysis and/or functional studies on collagen XVII.

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Figure 1. Phenotypic variability in junctional epidermolysis bullosa (JEB) patients with *COL17A1* mutations. (a) dystrophic nails. (b) dental abnormalities. (c) congenital cutis aplasia localized in four limbs. (d) blister in an upper limb.

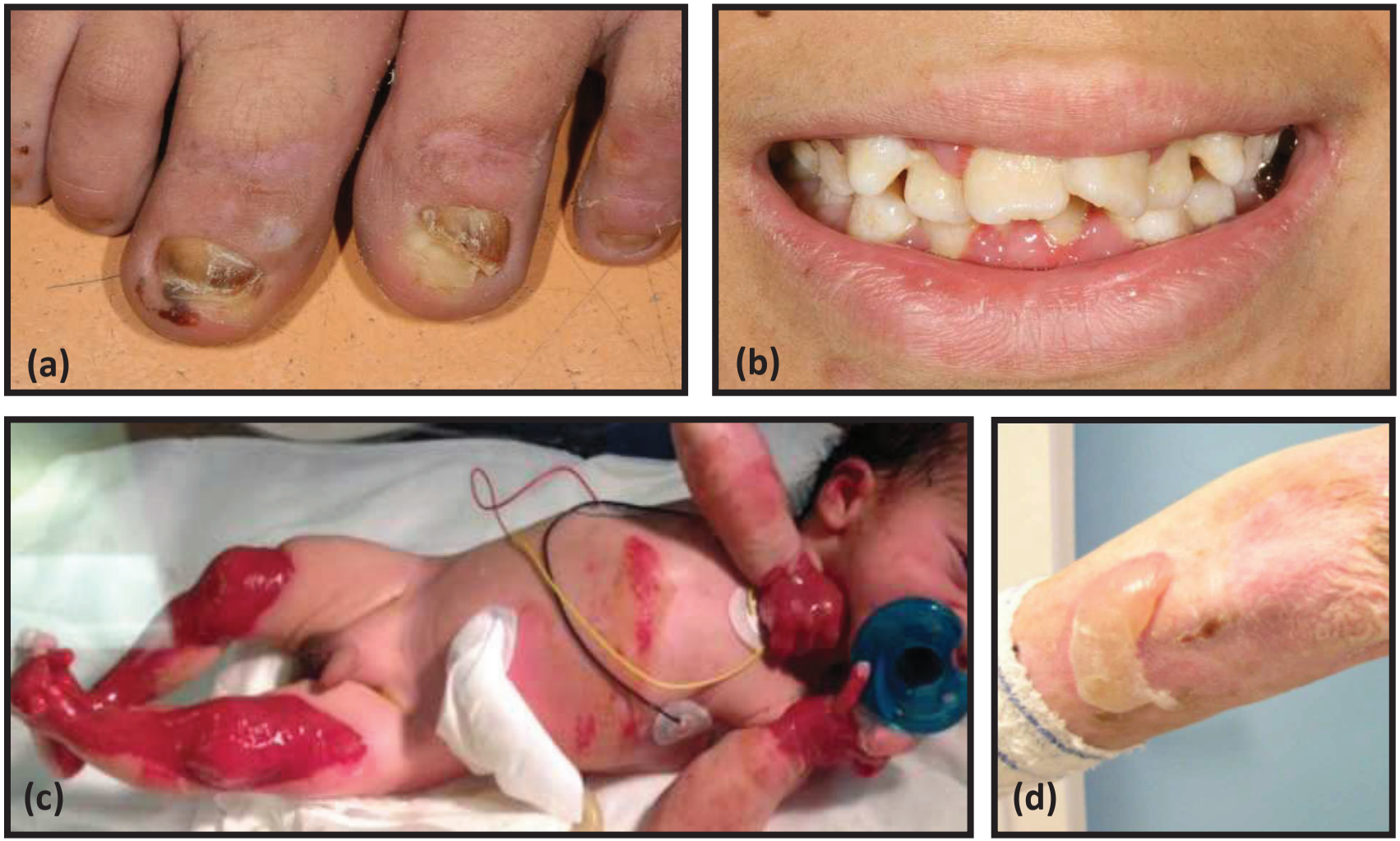

Figure 2. Distribution of collagen XVII mutations in this study. Schematic representation of the protein with corresponding domains and binding site proteins. The mutations underlined are novel, asterix corresponds to homozygous mutations and recurrent mutations are grayed. Nonsense and missense mutations are described on protein level, whereas frameshift, splice site, deletion and insertion mutations are described on cDNA level.

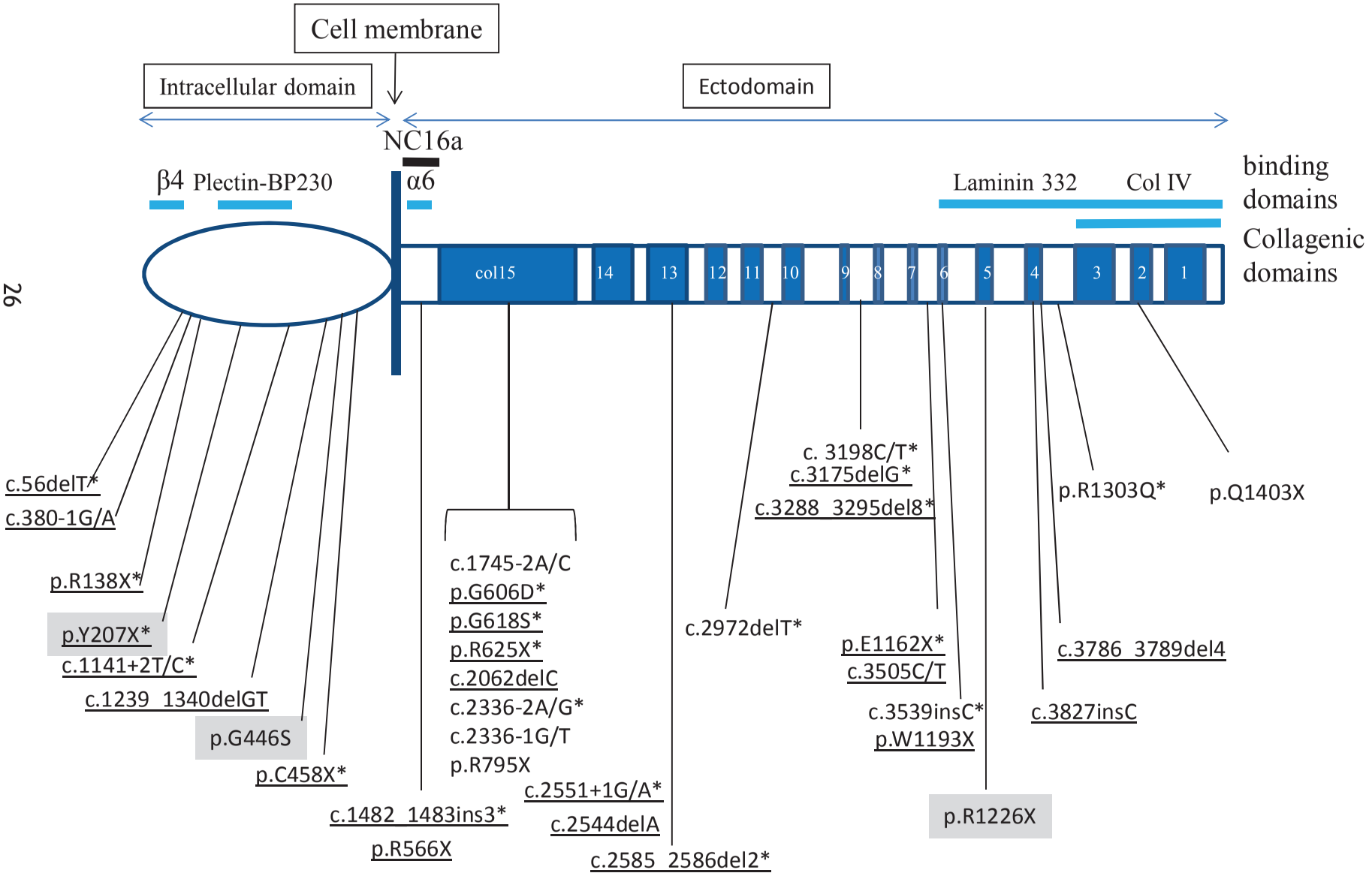

Table 1. Summary of clinical, immunohistological and molecular data of patients. X: Premature terminal codon. SS: Splice site mutation. A: Alanine. E: Acide glutamique. G: Glycine. Y: Tyrosine. L: Leucine. Q: Glutamine. R: Arginine. S: Serine. NA: Non Available. PMD: Psycho motor delay. Ins : Insertion. Del: Deletion. Mut : Mutation. Prot: Protein. Pb: Problems.

| Patients | Age | mut cDNA 1 | mut prot 1 | exon 1 | mut cDNA 2 | mut prot 2 | exon 2 | IF n=29 | ACC n=25 | Alopecia n=25 | Mucosa lesions n=25 | Nails pb n=25 | Dental pb n=25 | Evolution n=25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **JEB late onset** | | | | | | | | | | | | | |
| 1 | 23 | c.3908G>A | p.R1303Q | 52 | c.3908G>A | p.R1303Q | 52 | - | no | no | oral | yes | no | good |
| | **JEB localized** | | | | | | | | | | | | | |
| 2 | 71 | c.1852 G/A | p.G618S | 23 | c.1852 G/A | p.G618S | 23 | ± | no | yes | urogenital | no | no | good |
| 3 | 17 | c.2383C/T | p.R795X | 33 | c.1239_1340delGT | p.V413fs | 16 | - | no | no | oral/uro-genital | yes | yes | good |
| | **JEB generalized** | | | | | | | | | | | | | |
| 4 | NA | c.1873C/T | p.R625X | 23 | c.1873C/T | p.R625X | 23 | - | 4 limbs | yes | oral/uro-genital | yes | no | demise at 1month/meningitis |
| 5 | NA | c.56delT | p.V19fs | 3 | c.56delT | p.V19fs | 3 | - | 4 limbs | yes | oral/uro-genital | yes | no | demise at 1month/renal failure |
| 6 | NA | c.2062delC | p.R688fs | 26 | c.3676C/T | p.R1226X | 51 | - | 4 limbs | yes | oral/uro-genital/orl | yes | no | demise at 3month/sepsis |
| 7 | 3 | c.412C/T | p.R138X | 7 | c.412C/T | p.R138X | 7 | - | no | yes | oral/orl/eyes | yes | yes | malnutrition/PMD/retraction |
| 8 | 2 | c.3505C/T | p.R1169X | 49 | c.2544delA | p.G848fs | 36 | - | 4 limbs | yes | oral/urogenital | yes | yes | PMD |
| 9 | 2 | ongoing | | | ongoing | | | - | lower limbs | yes | oral/orl/eyes | yes | no | malnutrition/PMD/retraction |
| 10 | 30 | c.2585_2586del2 | p.P862R | 37 | c.2585_2586del2 | p.P862R | 37 | - | lower limbs | yes | oral/uro-genital/eyes | yes | yes | malnutrition/retraction |
| 11 | 54 | c.621 C/G | p.Y207X | 10 | c.621 C/G | p.Y207X | 10 | - | no | yes | oral/urogenital/orl | yes | yes | depression |
| 12 | 22 | c.380-1G>A | SS | 7 | c.1696C/T | p.R566X | 19 | - | lower limbs | yes | oral/urogenital/eyes | yes | yes | malnutrition/depression |
| 13 | 2 | c.3539insC | p.P1180fs | 50 | c.3539insC | p.P1180fs | 50 | - | 4 limbs | no | oral/uro-genital | yes | no | malnutrition |
| 14 | 0 | c.3579G/A | p.W1193X | 50 | c.3786_3789del4 | p.F1262fs | 52 | - | 4 limbs | no | oral/nose | yes | no | heavy local care |
| 15 | 0 | c.621C/G | p.Y207X | 10 | c.621 C/G | p.Y207X | 10 | ± | upper limbs | no | oral | no | no | heavy local care |
| 16 | 17 | c.1482_1483ins3 | ins 1AA | 18 | c.1482_1483ins3 | ins 1AA | 18 | ± | 4 limbs | yes | oral/eyes | yes | no | good |
| 17 | 9 | c.1373C/A | p.C458X | 17 | c.1373C/A | p.C458X | 17 | - | 4 limbs | yes | oral | yes | yes | good |
| 18 | 13 | c.3487G/T | p.E1163X | 49 | c.3487G/T | p.E1163X | 49 | - | 4 limbs | yes | oral/orl | yes | yes | good |
| 19 | 11 | c.3827insC | p.P1276fs | 52 | c.3676C/T | p.R1226X | 51 | - | 4 limbs | yes | oral | yes | yes | good |
| 20 | 32 | c.1336G/A | p.G446S | 17 | c.1745-2A>C | SS | 21 | - | no | yes | oral/urogenital/nose | yes | yes | good |
| 21 | 16 | c.3175delG | p.E1059fs | 46 | c.1336G/A | p.G446S | 17 | - | no | no | oral/urogenital | yes | yes | good |
| 22 | 25 | c.2551+1G>A | SS | 36 | c.2551+1G>A | SS | 36 | - | no | no | oral | yes | yes | good |
| 23 | 17 | c.2551+1G>A | SS | 36 | c.2551+1G>A | SS | 36 | - | no | no | oral | yes | yes | good |
| 24 | 13 | c.3198C/T | SS | 46 | c.2972delT | p.V991fs | 45 | ± | no | no | oral | yes | yes | good |
| 25 | 74 | NF | NF | NF | NF | NF | NF | ± | no | no | oral | yes | yes | good |
| 26 | 20 | c.1141+2T>C | SS | 14 | c.1141+2T>C | SS | 14 | - | NA | NA | | NA | NA | no info |
| 27 | 14 | c.1817G>A | p.G606D | 22 | c.1817G>A | p.G606D | 22 | - | NA | NA | | NA | NA | no info |
| 28 | 48 | c,2336-2A>G | SS | 32 | c,2336-2A>G | SS | 32 | NA | NA | NA | | NA | NA | no info |
| 29 | 20 | c.4207C/T | p.Q1403X | 53 | c.2336-1G>T | SS | 32 | - | NA | NA | | NA | NA | no info |
| 30 | 12 | c.3288_3295del8 | p.V1095fs | 48 | c.3288_3295del8 | p.V1095fs | 48 | - | NA | NA | | NA | NA | no info |

# SERMENT D'HIPPOCRATE

Au moment d'être admis(e) à exercer la médecine, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité.

Mon premier souci sera de rétablir, de préserver ou de promouvoir la santé dans tous ses éléments, physiques et mentaux, individuels et sociaux.

Je respecterai toutes les personnes, leur autonomie et leur volonté, sans aucune discrimination selon leur état ou leurs convictions. J'interviendrai pour les protéger si elles sont affaiblies, vulnérables ou menacées dans leur intégrité ou leur dignité. Même sous la contrainte, je ne ferai pas usage de mes connaissances contre les lois de l'humanité.

J'informerai les patients des décisions envisagées, de leurs raisons et de leurs conséquences. Je ne tromperai jamais leur confiance et n'exploiterai pas le pouvoir hérité des circonstances pour forcer les consciences. Je donnerai mes soins à l'indigent et à quiconque me les demandera. Je ne me laisserai pas influencer par la soif du gain ou la recherche de la gloire.

Admis(e) dans l'intimité des personnes, je tairai les secrets qui me seront confiés. Reçu(e) à l'intérieur des maisons, je respecterai les secrets des foyers et ma conduite ne servira pas à corrompre les moeurs.

Je ferai tout pour soulager les souffrances. Je ne prolongerai pas abusivement les agonies. Je ne provoquerai jamais la mort délibérément.

Je préserverai l'indépendance nécessaire à l'accomplissement de ma mission. Je n'entreprendrai rien qui dépasse mes compétences. Je les entretiendrai et les perfectionnerai pour assurer au mieux les services qui me seront demandés.

J'apporterai mon aide à mes confrères ainsi qu'à leurs familles dans l'adversité.

Que les hommes et mes confrères m'accordent leur estime si je suis fidèle à mes promesses ; que je sois déshonoré(e) et méprisé(e) si j'y manque.

# ABSTRACT

**Background:** Junctional epidermolysis bullosa (JEB) associated with *COL17A1* genetic mutations is a rare genodermatosis characterized by skin and mucosal fragility due to impaired expression of collagen type XVII, an adhesion protein component of the hemidesmosomes. Clinical phenotypes span from severe forms causing early demise to late onset associated with limited involvement.

**Objective:** We aimed at defining the genotypes of a large cohort of JEB patients with *COL17A1* mutations.

**Methods:** Diagnoses were first established by immunofluorescence analyses of skin biopsies followed by genetic screening for *COL17A1* mutations between June 1993 and July 2015.

**Results:** A wide panel of phenotypes including lethal and mild late-onset forms was observed in the 30-patient cohort. Fourteen of 25 patients also presented with extensive congenital aplasia cutis, while in 13/25 patients involvement improved along with age at the skin but not at the mucosa level. Residual staining of collagen XVII in skin correlated with favorable prognoses. Of the 34 *COL17A1* mutations identified, 24 are novel. Three of them (p.Arg1226X, exon 51; p.Gly446Ser, exon 17; p.Tyr207X exon 10) are recurrent in European patients. If missense and splice site mutations correlated well with mild phenotypes, no other clear phenotype-genotype relationship could be established.

**Discussion:** We report novel clinical aspects of JEB associated with *COL17A1* genetic mutations. Except for recurrent mutations, a genotype-phenotype correlation is difficult to establish and needs mRNA analysis and/or functional studies.

**Key words:** Epidermolysis Bullosa ; Collagen XVII ; *COL17A1* ; Cutis Congenital aplasia ; phenotype.

# RESUME

**Introduction:** L'épidermolyse bulleuse héréditaire de type jonctionnelle (JEB) associée à des mutations du gène *COL17A1* est une génodermatose rare caractérisée par une fragilité cutanéomuqueuse due à une altération de l'expression du collagène de type XVII, un composant des protéines d'adhésion des hémidesmosomes. Le phénotype varie de formes graves entrainant une mort prématurée à des formes tardives avec une atteinte cutanéomuqueuse limitée.

**Objectif:** Nous avons cherché à définir le profil génotypique et phénotypique d'une grande cohorte de patients atteints de JEB et présentant des mutations du gène *COL17A1*.

**Méthodes:** Le diagnostic a été d'abord établi par analyse des biopsies cutanées par immunofluorescence suivie d'une recherche génétique des mutations du gène *COL17A1*.

**Résultats:** Une grande variété de phénotypes, allant des formes létales aux formes bénignes, a été observée dans notre cohorte de 30 patients. Quatorze patients sur 25 présentaient une vaste aplasie cutanée congénitale, tandis que 13 patients sur 25 présentaient une atteinte cutanée d'évolution favorable avec l'âge, sans amélioration de l'atteinte muqueuse. La coloration résiduelle du collagène XVII observée lors de l'immunofluorescence indirecte était corrélée à un pronostic favorable. Sur les 34 mutations du gène *COL17A1* identifiées, 24 étaient nouvelles. Trois d'entre elles (p.Arg1226X, exon 51; p.Gly446Ser, exon 17; p.Tyr207X exon 10) étaient récurrentes chez nos patients européens. Si les mutations faux-sens et les mutations au niveau des sites d'épissage sont bien corrélés avec des phénotypes modérés, aucune autre corrélation génotypique-phénotypique n'a pu être clairement établi.

**Discussion:** Nous rapportons de nouveaux aspects cliniques des JEB associées aux mutations du gène *COL17A1*. À l'heure actuelle et à l'exception des mutations récurrentes extrêmement rares, une corrélation génotypique-phénotypique valide ne peut pas être établie. Ces corrélations nécessitent l'identification de cas supplémentaires de JEB.

**Mots clés :** Epidermolyse bulleuse ; Collagène XVII ; *COL17A1* ; aplasie cutanée congénitale ; phénotype.